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Understanding the genetics of regulation of aflatoxin production and *Aspergillus flavus* development

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Abstract

Aflatoxins are polyketide-derived, toxic, and carcinogenic secondary metabolites produced primarily by two fungal species, *Aspergillus flavus* and *A. parasiticus*, on crops such as corn, peanuts, cottonseed, and treenuts. Regulatory guidelines issued by the U.S. Food and Drug Administration (FDA) prevent sale of commodities if contamination by these toxins exceeds certain levels. The biosynthesis of these toxins has been extensively studied. About 15 stable precursors have been identified. The genes involved in encoding the proteins required for the oxidative and regulatory steps in the biosynthesis are clustered in a 70 kb portion of chromosome 3 in the *A. flavus* genome. With the characterization of the gene cluster, new insights into the cellular processes that govern the genes involved in aflatoxin biosynthesis have been revealed, but the signaling processes that turn on aflatoxin biosynthesis during fungal contamination of crops are still not well understood. New molecular technologies, such as gene microarray analyses, quantitative polymerase chain reaction (PCR), and chromatin immunoprecipitation are being used to understand how physiological stress, environmental and soil conditions, receptivity of the plant, and fungal virulence lead to episodic outbreaks of aflatoxin contamination in certain commercially important crops. With this fundamental understanding, we will be better able to design improved non-aflatoxigenic biocompetitive *Aspergillus* strains and develop inhibitors of aflatoxin production (native to affected crops or otherwise) amenable to agricultural application for enhancing host-resistance against fungal invasion or toxin production. Comparisons of aflatoxin-producing species with other fungal species that retain some of the genes required for aflatoxin formation is expected to provide insight into the evolution of the aflatoxin gene cluster, and its role in fungal physiology. Therefore, information on how and why the fungus makes the toxin will be valuable for developing an effective and lasting strategy for control of aflatoxin contamination.

Key words: aflatoxins, *Aspergillus flavus*, gene cluster, genomics, mycotoxins, secondary metabolite

Introduction

Mycotoxins are fungal metabolites that are capable of producing acute toxic or chronic carcinogenic, mutagenic, teratogenic, or estrogenic responses in higher vertebrates and other animals [1–3]. Within the last decade, significant advances have been made in mycotoxin detection methods and control strategies as well as in understanding the biochemistry, genetics and regulation of mycotoxin biosynthesis. The biosynthetic pathways for mycotoxins such as aflatoxins, fumonisms and trichothecenes, the clustering of biosynthetic genes, and the functions of these genes have been elucidated in great detail [4–8]. Despite the many advances described above, mycotoxin contami-

nation problems are far from being solved. There remains a vast gap in our understanding of the coordinated global regulation of toxin formation, of the signal transduction pathways underlying primary and secondary metabolisms, of the biotic and abiotic factors that affect toxin formation, and of the interactions of mycotoxigenic fungi and their host plants during infection. Those that significantly impact agriculture include aflatoxins AF produced by *A. flavus* and *A. parasiticus*; Zearalenone and the trichothecenes (in particular deoxynivalenol) produced by *Fusarium spp.;* ochratoxins produced by *A. ochraceus* and *Penicillium viridicatum*; and fumonisins produced by *Fusarium verticillioides* [4, 8]. Cyclopiazonic acid (CPA) produced by *A. flavus*, may also be of concern. The FDA has estimated direct economic losses of nearly a billion dollars a year due to crop loss, and another half billion dollars in mitigating costs due to the impact of only three mycotoxins, namely aflatoxins, fumonisins, and trichothecenes [3]. Aflatoxins, potent toxins, and carcinogens are the most widely studied of all mycotoxins [2, 9, 10]. It is not surprising then that the most significant research progress towards controlling mycotoxins has been made with aflatoxins. Aflatoxins frequently contaminate agricultural commodities and thus pose serious health hazards to both humans and domestic animals [3, 11]. Seventy-seven countries are known to have regulations limiting mycotoxin levels with 48 having specific regulatory levels for total aflatoxins in foodstuffs and 21 having regulations for aflatoxins in feedstuffs [12]. The FDA has set limits of 20 ppb total aflatoxins for interstate commerce of food and feed and 0.5 ppb of aflatoxin M_1 for sale of milk.

The most likely processes by which crops are infected by *A. flavus* and contaminated by aflatoxin have been reviewed [13–15]. Elimination of pre-harvest aflatoxin contamination through the development of novel biotechnological control strategies [16–18] could benefit significantly by additional knowledge of the fundamental molecular and biological mechanisms that regulate the biosynthesis of aflatoxin by the fungus; fungal survival in the ecosystem and its ability to invade crops [5, 10, 19]. The rapid development of high throughput sequencing made it possible in genetic research to advance from single gene cloning to whole genome sequencing. The technological breakthrough allows scientists to study the genome of an organism possibly in a very short time frame compared with traditional genetic studies. Tremendous advances have also been made in understanding the genetics of four non-aflatoxigenic *Aspergillus* species, *A. oryzae*, *A. sojae*, *A. niger* and *A. fumigatus*. The three former species are economically important because of their industrial applications. For example, *A. oryzae* and *A. niger* are used in the production of enzymes, peptides and other organic compounds, and *A. sojae* is used in the fermentation of soy sauce, which is a billion dollar industry worldwide. In contrast, *A. fumigatus* is a human and animal pathogen and is the most common cause worldwide of human aspergillosis, an often-fatal disease that affects primarily immunocompromised individuals. Currently, the whole genome sequencing and/or Expressed Sequence Tag (EST) projects for *A. flavus* have been completed.

Genetics of aflatoxin biosynthesis

Previous studies determined that aflatoxins are synthesized by a polyketide metabolic pathway [5, 7, 10, 20]. Mapping of overlapping cosmid clones of *A. parasiticus* and *A. flavus* genomic DNA established that the genes in the aflatoxin biosynthetic pathway are clustered [5, 7, 21]. In general, the aflatoxin gene cluster in *A. parasiticus* and *A. flavus* consists of 25 genes spanning approximately 70 kb (Figure 1). A positive regulatory gene, *aflR*, coding for a sequence-specific, zinc-finger DNA-binding protein is located in the cluster and is required for transcriptional activation of most, if not all, of the aflatoxin structural genes [5]. Adjacent to and divergently transcribed from the *aflR* gene is *aflJ*. *aflJ* has not demonstrated significant homology with any other genes/proteins present in databases. Though the exact function of *aflJ* is not clear at this time, it has been shown to be necessary for expression of other genes in the aflatoxin cluster [22, 23]. The function of most of the aflatoxin gene products has been deduced either by genetic or biochemical means [7, 24–27]. Of the 25 genes identified in the pathway, only four (*norA, norB, aflT*, and *ordB*) have yet to have the function of their protein product determined experimentally.

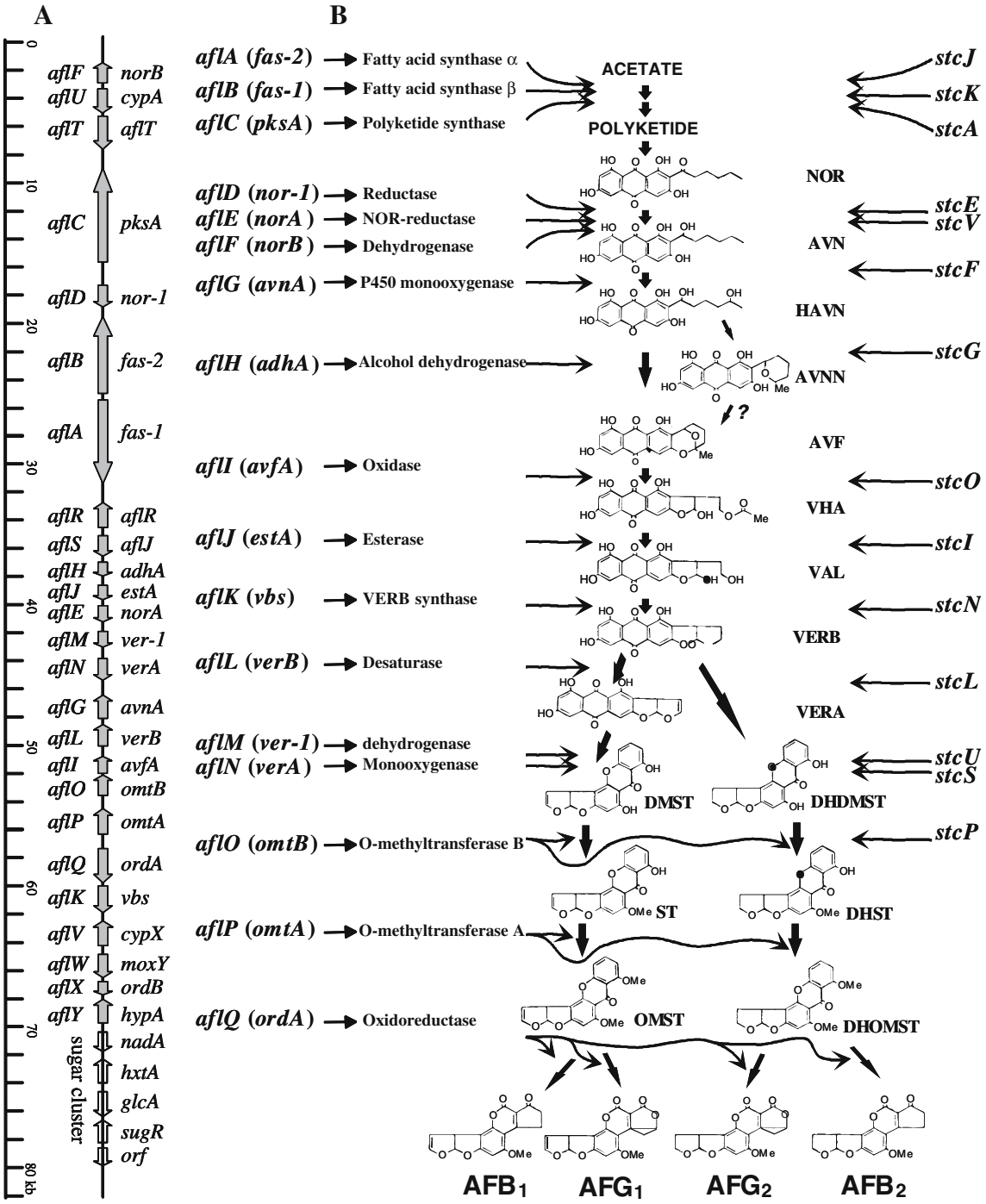

Figure 1. The gene cluster responsible for aflatoxin biosynthesis in *Aspergillus flavus* and *A. parasiticus*. (A) Clustered genes (arrows indicate the direction of gene transcription) and (B) the aflatoxin biosynthetic pathway. The ST biosynthetic pathway genes in *A. nidulans* are indicated at the right of panel B. Arrows in panel B connect the genes to the proteins they encode. Abbreviations: NOR, norsolorinic acid; AVN, averantin; HAVN, 5′-*hydroxy-averantin; OAVN, oxoaverantin; AVNN, averufanin; AVF, averufin; VHA, versiconal hemiacetal acetate; VAL, versiconal; VERB, versicolorin B; VERA, versicolorinA; DMST, demethylsterigmatocystin; DHDMST, dihydrodemethylsterigmatocystin; ST, sterigmatocystin; DHST, dihydrosterigmatocystin; OMST, O-methylsterigmatocystin; DHOMST, dihydro-O-methylsterigmatocystin; AFB$_1$, aflatoxin B$_1$; AFB$_2$, aflatoxin B$_2$; AFG$_1$, aflatoxin G$_1$; and AFG$_2$, aflatoxin G$_2$* [7].

The mycotoxin ST is an aflatoxin precursor. In some fungi more distantly related to *A. flavus* and *A. parasiticus*, ST is the final metabolite. The biosynthetic and regulatory genes required for ST production in *A. nidulans* are homologous to those required for aflatoxin production in *A. flavus* and *A. parasiticus* and they also are clustered [7, 28]. However, the organization of the genes in the *A. nidulans* cluster is quite different from that in *A. parasiticus* and *A. flavus*. The sequence identity of the clustered genes between *A. parasiticus* and *A. flavus* is about 90–99%, but between *A. parasiticus* and *A. nidulans* is 55-75% [7].

Transcriptional regulation of aflatoxin synthesis

Most of the 25 genes in the aflatoxin biosynthetic pathway gene cluster (Figure 1) are regulated by a Gal4-type 47 kDa C6-zinc binuclear cluster DNA-binding protein, AflR [5]. AflR binds to the palindromic motif 5′-TCGN$_5$CGA-3′ in the promoter region of aflatoxin structural genes. The promoter regions of the majority of aflatoxin genes have at least one 5′-TCGN$_5$CGA-3′ binding site within 200 bp of the translation start site, though some putative binding sites have been identified further upstream [7, 19]. Based on comparison of 16 possible sites, the consensus binding sequence was 5′-TCGSWVNSCGR-3′ [29, 30]. By analogy to most Gal4-type proteins that bind to partially palindromic sites, AflR probably binds to its recognition site as a dimer. The gene, *aflR* may be self-regulated, as well as, under the influence of negative regulators [31–35]. Upstream elements may be involved in negative regulation of promoter activity of *aflR* [34].

A number of studies have identified a genetic connection between aflatoxin/sterigmatocystin biosynthesis and fungal development [36–41]. *Aspergillus* developmental mutants have been used to partially elucidate the signaling pathway linking fungal secondary metabolism and development. The early work of Kale et al. [36, 37] recognized that mutants defective in conidiation often lost the ability to produce aflatoxin. This work was extended by characterizing a G-protein-mediated signaling cascade in *A. nidulans* that regulates both asexual sporulation and ST production [42] and the roles of cAMP and protein kinase A in both processes [39–41]. A possible transcription regulatory gene, *veA*, has been identified in *A. nidulans* and *A. parasiticus* that controls both toxin production and sexual development [43, 44]. Both *A. nidulans* and *A. parasiticus veA* mutants fail to produce ST or aflatoxin. In addition, *A. nidulans* and *A. parasiticus* do not produce cleistothecia (sexual fruiting bodies harboring ascospores) and sclerotia (asexual overwintering structures), respectively. No significant sequence identity has been found between *veA* and other genes present in databases. Lastly, a number of genetic loci were identified in *A. nidulans* mutants that resulted in loss of ST production but had normal developmental processes [45]. Complementation studies with one of these mutants identified a gene, *laeA*, that encodes an enzyme with sequence similarity to methyltransferases and appears to be required for expression of ST. *LaeA* homologs have been found in a number of filamentous fungi. In all species examined, disruption of *laeA* resulted in loss of secondary metabolite production while overexpression of *laeA* results in hyperproduction of the secondary metabolite [40].

Chromosomal location also affects expression of genes in the aflatoxin cluster [46]. Previous studies have provided evidence that epigenetic factors also affect the developmental changes necessary for the fungus to be able to switch from normal vegetative growth (primary metabolism) to secondary metabolism (aflatoxin production) and reproductive cells (sclerotia and conidia) [40, 42, 44, 47, 48]. The subtelomeric location of the aflatoxin gene cluster on chromosome 3 may affect the accessibility of cluster genes to transcription factors. Chromosomal location has been shown to affect expression of aflatoxin biosynthetic genes [46, J. Yu, unpublished results). Boundary elements near telomeres separate active from inactive chromatin domains and stop the spread of inactive chromatin. Inactive chromatin has more methylation of lys-9 of histone H3 (H3-Me-K9) and less H3-K9 and H4-acetylation than that of active chromatin. Furthermore, globally acting transcription factors, including those mediating nitrogen, carbon, and pH regulation, affect chromatin organization near the site of transcription initiation [49]. More directly, they also act as transcription activating or inhibiting factors by binding to sequence-specific sites in the promoter and either complexing with other transcription

factors or by blocking the binding of positively acting transcription factors [50].

Effect of environmental factors on toxin synthesis

The most significant environmental factors that influence toxin synthesis are carbon and nitrogen sources, pH, temperature, water activity, and plant metabolites [5, 38]. Highest levels of aflatoxin are produced when the fungus invades the seed embryo, where the highest levels of simple sugars (glucose and sucrose) are present compared to other parts of the seed, where complex carbohydrates predominate [51]. Aflatoxin biosynthesis has been known to be induced by simple carbohydrates, such as glucose and sucrose (reviewed in 52], but not by peptone or complex sugars such as starch. Interestingly, a hexose utilization gene cluster (Figure 1) is located adjacent to the aflatoxin biosynthetic cluster and may affect expression of aflatoxin genes [53]. Nitrogen supply is usually not a limiting factor for U.S. crop cultivation [15], but pools of amino acids in the plant, dependent on nitrogen, are reported to be important in regulation of toxin formation [54–56]. Nitrate suppression of aflatoxin synthesis in some *Aspergillus* isolates has been well documented [57–59], whereas nitrogen supplied as ammonium in media supports toxin formation. The cause of the nitrate inhibitory effect is still uncertain, but nitrate inhibition could be mediated by the globally acting nitrogen source transcription factor AreA [34, 35, 60]. AreA binds to the tetranucleotide recognition site, GATA, in the promoters of *aflR* and *aflJ* and affects their expression [29]. Certain strains of *A. flavus* respond differently to nitrate than do other strains, and these differences correlate with differences in the number of GATA sites near the *aflJ* transcription start site [61].

Fungi are capable of growing over a wide pH range. Under drought stress, physiological pH shifts in plants could occur [62, 63]. It has been established that aflatoxin synthesis optimally occurs in the pH range of 3.4–5.5. Regulation of fungal metabolism by ambient pH involves a globally acting transcription factor encoded by *pacC* that is post-synthetically modified by a pH-sensing protease [64, reviewed in 65]. A number of PacC-binding sites are located in the promoter regions of some aflatoxin biosynthesis genes and could be involved in their negative regulation at basic pH [29, 66]. Neither high temperature nor drought stress alone will lead to increased concentrations of aflatoxin [67]. It is not known whether these factors are conducive for the fungus to initiate the infection process in crops. High maximum and high minimum daily temperatures, especially during periods of high net evaporation, are more important for the development of aflatoxin than humidity or average precipitation during the same period. The ideal temperature for aflatoxin production is 29–30 °C [15, 68]. Aflatoxin production is significantly decreased at temperatures below 25 °C, but is completely inhibited at 37 °C or above. Drought stress can increase the percentage of seed infected. The higher the water activity, the better are the conditions for fungal growth and toxin synthesis. At water activity below 0.85, the growth of the fungus and its spore germination rates are considerably slowed [15]. At water activity between 0.70–0.75, growth and spore germination cease.

Natural plant metabolites affect toxin production and fungal development

Earlier research efforts in this, as well as other labs, have shown that plant volatiles can alter either *Aspergillus* growth or aflatoxin production, i.e., volatile aldehydes and other compounds from neem leaf [69, 70], cotton leaf [71] and corn-leaf [72]. Anthocyanins and related flavonoids also affect aflatoxin biosynthesis [73]. In some cases, growth was not significantly affected by various metabolites, while aflatoxin biosynthesis and fungal development were significantly decreased [71, 74–77].

The plant oxylipins 13-hydroperoxylinoleic acid and 9-hydroperoxy linoleic acid, as well as their precursor, linoleic acid, affect sexual and asexual sporulation in *A. nidulans*, sclerotial development, and toxin synthesis [78–82]. The conversion of oleic acid (18:1) to linoleic acid (18:2) is a critical biosynthetic step in the generation of sporogenic psi factors. This conversion is mediated by a delta-12 desaturase. Calvo *et al.* [83] identified and cloned the delta-12 desaturase (*odeA*) gene from *A. nidulans* and *A. parasiticus*. An *A. parasiticus odeA* mutant demonstrated delayed spore germination, a twofold reduction

Table 1. A. flavus gene (EST) ontology assignments [87]*

Molecular Function	1015 TC/singleton
Enzyme	401
Binding	231
Transporter	110
Structural molecule	74
Molecular function unknown	65
Signal transducer	33
Transcription regulator	24
Translation regulator	23
Obsolete molecular function	18
Chaperone	15
Enzyme regulator	13
Cell adhesion molecule	3
Defense/immunity protein	2
Protein tagging	1
Motor	1
Apoptosis regulator	1
Cellular Component	1378 TC/singleton
Cell	1278
Cellular component unknown	72
Extracellular	15
Unlocalized	7
Obsolete molecular function	4
Cell wall	2
Biological Process	2376 TC/singleton
Cell growth and/or maintenance	2090
Cell communication	169
Development	38
Obsolete molecular function	31
Biological process unknown	19
Death	13
Physiological processes	12
Behavior	4

*The identified unique ESTs were blasted against a non-redundant protein database. The classification of the molecular functions are shown in this table.

in growth, a reduced level of conidiation, and complete loss of sclerotial development compared to the wild-type fungus [84]. It is possible that the sporogenic properties of linoleic acid and hydroperoxylinoleic acids occur through conversion of these molecules into psi factor or by interfering with and/or mimicking psi factor due to high structural similarity between these 18:2 fatty acids.

Aflatoxin biosynthesis is inhibited by gallic acid, generated from hydrolysis of tannic acid in tree nuts [85]. During invasion by *A. flavus*, fungal tannase releases gallic acid from the tannic acid present in the pellicle of walnut and the hull of pistachio. Gallic acid is an antioxidant and may suppress some of the oxidative steps required for aflatoxin biosynthesis [86].

Effects of AflR as a positive regulator of aflatoxin biosynthesis, as well as the effects of plant metabolites and their environmental and nutritional factors on fungal development, are beginning to be understood. However, there is still much that we do not know about the mechanisms by which global regulatory genes and signaling pathways control aflatoxin gene expression and fungal development, particularly during crop invasion. These answers can be rapidly obtained through genomics of the fungus.

Genomics

Aspergillus flavus genomics is aimed at understanding the genetic control and regulation of toxin production by this important aflatoxigenic fungus as well as the evolutionary process in *Aspergillus* section *Flavi*. The relationship between toxin production and its survival, the relationship between primary and secondary metabolism, and the regulation and coordination of aflatoxin formation by potential regulator(s) upstream of *aflR* are important factors to understand in order to control aflatoxin contamination. More importantly, we need to understand what the mechanism of toxin production is in response to environmental conditions like nutrition status of crops, temperature, water stress, pH, and volatile compounds from plants.

The complete *A. flavus* expressed sequence tag (EST) sequence, DNA microarrays based on this sequence, and the entire sequence of the *A. flavus* genome are now available. The Institute for Genomic Research (TIGR), in conjunction with the Food and Feed Safety (FFS) Unit of the USDA-ARS, Southern Regional Research Center (SRRC), sequenced 26,000 clones (GenBank accession #: CO133039 to CO152656) and identified 7218 unique ESTs [87]. Thirty percent of the ESTs had related sequences in the GenBank Database. The fully annotated EST data set was released to the public by Gene Index constructed at TIGR (http://www.tigr.org/tigr-scripts/tgi/T index.cgi?species = a_*flavus*). Annotation has identified many genes that are potentially involved directly or indirectly in aflatoxin production

(global regulation, signal transduction, pathogenicity, virulence, and fungal development; 87, Table 1).

Gene profiling using microarrays is a powerful tool to detect a whole set of genes transcribed under specific conditions and to study the biological functions of these interested genes, gene expression and regulation, and to identify factors involved in plant-microbe (crop-fungus) interaction. DNA microarrays have been constructed at TIGR for functional studies of *A. flavus* biology. The high density and high quality microarray of 6684 short amplicons representing 5002 unique gene elements include the 31 known aflatoxin cluster genes. Gene expression profiling experiments by microarrays have been used by our group and others to successfully identify differentially-expressed genes associated with aflatoxin production in *A. flavus* and *A. parasiticus* grown under different nutritional and environmental conditions [87–90]. Near-isogenic developmental and secondary metabolic mutants of *A. flavus* and *A. parasiticus* are being used in microarray studies that will not require shifts in growth media. This will allow us to more accurately select specific subsets of differentially-expressed genes that are involved in aflatoxin production and fungal development.

The FFS Research Unit of SRRC is also participating in a national project to sequence the entire *A. flavus* genome spearheaded by Dr. Gary Payne of North Carolina State University, with the sequencing being done at TIGR (Dr. W. Nierman leading the effort). Karyotyping studies by CHEF gel had earlier indicated that there are about 6–8 chromosomes in the *A. flavus* genome ranging in size from 3 to ≥ 7 Mb each [91, 92]. By referencing of the karyotyping data of *A. oryzae*, [which contains 8 chromosomes, 93], a close relative of *A. flavus* or the domesticated strain of *A. flavus* as some scientists claim, it appears that there are most likely 8 chromosomes in *A. flavus*. The estimated genome size is about 33–36 Mb containing approximately 10,000–12,000 functional genes. Based on our research experiences with *Aspergillus*, the *A. flavus* genome is compacted with less duplicated sequence or multiple copies of genes. The non-coding sequences between genes are much shorter than in higher plants and contain only a few small introns within each gene, if any. The 36.2 Mb *A. flavus* genome sequence data are available to the public at the TIGR web site (http://www.tigr.org) and at http://www.aspergillusflavus.org.

Toxigenic potential of other *Aspergillus* spp

Aspergillus flavus and *A. parasiticus* are not known to reproduce sexually, and isolates of different vegetative compatibility groups (VCGs) cannot recombine, although phylogenetic incongruence based on DNA sequence alignments has been regarded as evidence that genetic recombination can occur [94–96]. The frequency of such events, if confirmed, is unknown.

Until recently, there have been very few verifiable reports of aflatoxin production by fungi other than *A. flavus*, *A. parasiticus*, and *A. nomius*. Studies have shown that an aflatoxin gene cluster is present in some strains of *A. oryzae* and *A. sojae* [97–100]. However, the genes in the two species are not transcribed [98, 100–103]. There are a number of *Aspergillus* species that produce aflatoxin but are not classified as section *Flavi* [for a detailed review see 104]. At present, these non-section *Flavi* isolates are not considered to be of significance with respect to contamination of agricultural crops. These studies could provide some insight into the evolutionary significance of aflatoxin production.

The use of biocompetitive agents to control toxin contamination

Microbial interference with aggressive non-aflatoxigenic *Aspergillus* is being developed as a way to reduce contamination of peanut, tree nuts, and cotton [105, 106]. The biocompetitive strains were chosen from atoxigenic *A. flavus* field isolates. However, efficacy of the competitor fungal strain could be improved through genetic engineering based on knowledge gained with understanding the molecular regulation of toxin synthesis. Fungal factors that determine the ability of an *A. flavus* strain to compete and overwinter in the soil, plant, and organic debris are still not known, and could be determined through fungal genomics and microarray analyses.

PCR-based identification of aflatoxigenic fungi

It is estimated that 25-50% of the crops harvested worldwide are contaminated with mycotoxins [107]. The serious health and economic consequences of mycotoxin contamination have created a need for rapid, sensitive, and reliable techniques to detect mycotoxins and mycotoxin producers within foodstuffs. A number of highly sensitive techniques have been developed for mycotoxin detection, including HPLC, GC-MS, and immunological-based assays. However, detection of mycotoxigenic fungi has relied, for the most part, on time consuming isolation and culturing techniques that require taxonomical expertise. The advent of a number of PCR-based techniques now allows for the rapid and reliable identification and quantification of fungi in foodstuffs [107–109]. Attempts to develop PCR-based methods for detection of aflatoxigenic fungi (*A. flavus* and *A. parasiticus*) are underway [110–113]. However, these methods utilize primers for aflatoxin genes that are not necessarily unique to aflatoxigenic fungi and have not been tested for reproducibility on a number of different contaminated commodities. Information gained from our studies on genomics of aflatoxigenic fungi will also allow for development of rapid, sensitive, and highly accurate method for detection of aflatoxigenic fungi present in a number of different, complex foodstuffs and also from soil samples.

It has been estimated that only about 25% of fungal species have been identified; therefore, it is probable that many fungi have not been discovered that possess some or all of the genes necessary for producing aflatoxin. We propose to utilize sequence information from the aflatoxin/ST genes of *A. flavus* and *Em. nidulans* in conjunction with sequence data that we are generating from aflatoxigenic, non-section *Flavi* isolates to design ''universal'' primers for PCR-based methods of identifying potential aflatoxigenic fungal isolates. Once these isolates are identified and shown to produce aflatoxin, studies of fungal ''fitness'' under adverse environmental conditions will be performed to determine if aflatoxin production provides an adaptive advantage for fungal survival and invasion of crops, particularly in view of the fact that many natural isolates of *A. flavus* do not produce aflatoxins [114].

Conclusion

An attempt has been made in this review to highlight that *A. flavus* genomics has provided researchers with a rapid and effective method for identification of genes potentially involved in aflatoxin formation and infection of crops by *A. flavus*. In addition, with the availability of the *A. oryzae* whole genome sequence a close relative of *A. flavus*, which is used in industrial fermentation for enzyme production that produce no aflatoxins, and of *A. fumigatus*, a human pathogen and a non-aflatoxin producer, these will be very helpful to identify genes specifically used by *A. flavus* for aflatoxin formation, for fungal invasion of crops and for fungal survival in the field through comparative genomics.

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